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REVIEW OF I. G. SHILLER'S BOOK
"DIRECTED ANTAGONISM OF MICROORGANISMS"

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Priority on a great number of discoveries in the science of microbiology rightly belongs to many of our compatriots who were active in this field. The great Russian scholar I. I. Mechnikov was the first one to express the idea of using the antagonism between microorganisms to suppress the putrescence microflora present in the human intestine.

This idea was taken up by the student and assistant of Mechnikov, I. G. Shiller, who worked out the basic principles and carefully investigated the problem of so-called forced or provoked antagonism between microorganisms. However, until recently, this extremely interesting problem has received very little attention.

I. G. Shiller is the author of about thirty published scientific works devoted to a many-sided elaboration of the problem of forced antagonism between microorganisms. All this great wealth of material is made available to the public in a book, which has just come out, which the author entitled "Directed Antagonism of Microorganisms" (Napravleny Antagonizm Mikrobov, Kiev, Ukrainian SSR State Medical Publishing House, 1952, 134 pages, 10,000 copies, 3 rubles 85 kopeks). This book records the outcome of investigations begun in 1914 in the laboratory of I. I. Mechnikov.

I. G. Shiller was the first person to elaborate a method for producing controlled antagonism between microorganisms, a method by means of which specific antibiotic substances derived from microorganisms can be obtained.

Shiller's works have been given a high evaluation by G. P. Kalina (1), who points out that they indicate a clear way of obtaining specific bacterial substances artificially, while maintaining the control necessary to make the substances useful for practical application. "The prospects for the use of the forced antagonism phenomenon", writes Kalina, "are very extensive, including even the possibility of exerting action on malignant tumors."

Shiller's book is made up of two parts: (a) a general section of four chapters, and (b) a specialized section of six chapters which comprises the principal part of the book. At the end of the book there is a short conclusion and a bibliography.

The author concentrated his attention on an analysis of the theoretical questions connected with the forced antagonism phenomenon. However, he gives a sufficiently detailed exposition of the practical utilization of the products of forced antagonism, and points out that therapy by means of them "has been found completely justified since 1930 in the field of dermatology and since 1937 in that of stomatology." (p 4)

In the present review our attention will be directed, mainly, to an analysis of the theoretical part of the problem, which we feel comprises the most important and interesting aspect of the work.

The nature of forced antagonism is as follows: bacteria which under natural conditions display no signs of antagonism are placed in an environment where there is a lack of nutritive substances (either nitrogenous or carbonaceous). One of the species of bacteria, the one having the ability to produce proteolytic enzymes, is then able to utilize bacteria of the other species as nutrient material.

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"The starting point for the first experiments," writes the author, "was the hypothesis that under conditions of nitrogen starvation, certain bacteria are capable of satisfying their own requirements for nitrogen at the expense of the cytoplasm of other microbes present with them in a medium, and of producing in this process specific bacteriolysins." (p 8)

By cultivating *Bac. mesentericus* and streptococcus cells in a starvation medium (distilled water or water-agar), the author achieved a development of *Bac. mesentericus* at the expense of the streptococcus cells which were dissolved by special lysins excreted by *Bac. mesentericus*.

Shiller succeeded in obtaining these lysins in a concentrated form by evaporating in a constant-temperature closet at 57° C the culture liquid in which the development of *Bac. mesentericus* had occurred. The culture liquid had previously been freed of the cells of *Bac. mesentericus*.

Upon the introduction of a dilute water solution of the previously concentrated lysins into a completely adequate nutrient medium containing fresh streptococcus cells, the latter undergo lysis.

It is a well-known fact that a living bacterial cell cannot be used by another microorganism as nutrient material until it has been killed. This is precisely the case under the conditions of forced antagonism: the living bacteria can be used by the nutrient antagonist only after they have been killed by the special antibacterial substances excreted into the surrounding medium. Shiller directed his attention to this capability, noting that "evidently microorganisms first kill the bacteria, and then utilize them as food by enzymatic action. Certain enzymes usually do not react upon living cells. Consequently, antibacterial substances that kill the bacteria are excreted first, and then the contents of the cell are used for food." (p 8)

From our point of view, this antibacterial substance excreted into the medium is of great interest since it is the fundamental factor in the whole forced antagonism process. It underlines the thought that the antibacterial substance plays a basic role in the antagonism process, since we are undoubtedly dealing mainly with a defensive function of the organism and not with the feeding process, which enters in only as a secondary and subordinate factor. This opinion is supported by the fact that forced antagonism may occur not only in a starvation medium but also in a completely adequate nutritional substrate.

In this regard, Shiller arrives at the completely justified conclusion that "forced antagonism of microorganisms can be identified with their defensive function and finds its expression in the excretion into the medium of bactericidal and bacteriolytic substances."

Sometimes digestive and defensive functions are taken care of by the same mechanism. This happens in a starvation medium. At other times, in media which contain nutrient elements or even in an animal organism (e.g., the intestine), these functions are separated. However, in both instances the bacteria react, by secretion, to the presence of other cells in the medium: they kill the other cells with the products thus excreted and dissolve them." (p 18)

Thus, we see that in the process of developing the forced antagonism phenomenon under conditions existing in a starvation medium, we can discern two functions in the activity of the microbe antagonist: (1) excretion into the surrounding medium of antibacterial substances which kill living bacterial cells, and (2) utilization of the contents of the killed cells as food material. The end result of the process is the multiplication of the active antagonist species. More will be said below about the relationship of these two functions to one another.

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A very valuable generalization in this theory is that the phenomenon is reversible. "This means," writes Shiller, "that the microorganism which has served in one instance as the source of food for another can, as a result of a change in its living conditions, in turn extract from the other microorganism substances necessary for its own nutrition." (p 58)

A relationship of forced antagonism, says Shiller, may occur not only between different species of bacteria, but also between bacteria and yeast or between microorganisms and individual animal tissues (animal cells). This last fact, in conjunction with the forced nourishment of microbes on various animal cells, is of special interest.

By cultivating proteolytic saprophytes on certain animal organs (for example, the heart muscle of a mouse) in a starvation medium, the author has shown that bacteria utilize animal tissue, excreting in the process lytic substances which produce a toxic reaction when they are introduced into an animal organism.

Through these experiments involving the utilization of animal tissues by microorganisms, precedents have been established, remarks Shiller, "for therapeutic experiments with malignant tumors." (p 113) Of great interest is the third chapter of the book, which is devoted to a discussion of forced antagonism and tuberculosis.

The principle of forced antagonism, as has already been indicated above, is that, under definite culture conditions, one microorganism can be transformed into the antagonist of another species and vice-versa. Basing his work on this principle, Shiller utilized, as the active antagonist of the tubercle bacillus, yeast cells which had been seeded into an aqueous solution of glucose together with a culture of tuberculosis bacilli. Under these conditions, the yeast cells began to utilize as their food, living cells of *M. tuberculosis*, excreting in this process the corresponding lysins.

"The digestion of tuberculosis bacilli by yeast cells," writes Shiller, "is of great theoretical as well as practical interest. From the theoretical point of view, adaptation of yeast cells so that they acquire the ability to excrete typical enzymes which can digest tuberculosis bacilli and which are absent in a pure culture, is worthy of attention. From the practical point of view, the digestion of tuberculosis bacilli by such an innocuous microorganism as the yeast cell can have therapeutic significance." (p67)

The proteolytic organisms, *Bac. subtilis*, *Bac. mesentericus*, *Bac. megaterium*, and others were also tried out as active antagonists to the tuberculosis bacillus. In a completely adequate nutritional medium, they form no substances which exert any influence on the tuberculosis bacillus. However, if a special cultural environment is created by putting them in a starvation medium together with *Mycobacterium tuberculosis* cells, they excrete substances which will dissolve the cells of the tuberculosis bacillus.

Using the principle of biological reversibility, the author cultivated tuberculosis bacilli on yeast cells in a starvation medium. In the course of this, the tuberculosis bacilli excreted substances which, in his opinion, approximated in their properties the toxic products formed in the organism. These substances are denoted as tuberculin. Their nature, unfortunately, has as yet not been investigated. On the basis of what has been explained here, it can be concluded that microorganisms under definite conditions of cultivation are able to alter the very nature of their own metabolism in such a way as to become adapted to new conditions of cultivation, thereby becoming capable of forming specific substances which were not peculiar to their former nature. Furthermore, the cited illustrations show that these substances can kill and dissolve the living cells of other species of organisms.

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Despite the wealth of factual material which the author has aptly presented, there are, unfortunately, certain errors in his work. The basic errors, from our point of view, are as follows:

1. Shiller, in our judgement, has incorrectly resolved the following question: At the expense of what substances -- in a medium in which there is a complete absence of nutritive material, i.e., in distilled water -- does the organism begin to develop in the first stages of its activities, and proceed to form, at the same time, specific lytic substances?

A correct solution of this problem of the forced antagonism theory would help us to penetrate much more deeply into the nature of the process which we are analyzing.

The author's hypothesis that a microorganism, upon coming in contact with cells of another species, immediately begins to excrete specific lytic substance, is difficult to accept.

It is a well-known fact that in order for a microorganism to begin to excrete any kind of a substance, this microorganism must be in an active functioning condition, i.e., it must receive nutrition and metabolize. Furthermore, for the formation of specific substances which are not peculiar to its own nature and which can kill and dissolve living bacterial bodies, a microorganism must, in the process of its development and perhaps not in one generation, change the character of its metabolism. All this is conceivable only in the course of the process of life activity which is connected with the utilization of those specific substances that take part in the anabolism and catabolism of the microorganism. Speculation about the presence of such substances in distilled water is not worthy of our consideration.

Although it is well-known that bacterial cells in distilled water can undergo a single or double cleavage evidently at the expense of their own reserve substances, the occurrence, in this instance, of a phenomenon which is so unusual for the organism in its natural state, i.e., the formation of specific metabolic products which kill and dissolve living cells of other species, is hardly possible.

The author of the book imagines that the development of the antagonist in the starvation medium is the result of merely one factor, i.e., the presence in the medium of organisms of another species. Shiller formulated this idea in the following manner: "In response to the presence in the medium of organisms of another species which provide an external stimulus, the division of microbes is accelerated." (p. 24) Such an approach to the developmental process of organisms appears to us to be one-sided and substantially erroneous. In this matter, consideration should be given to a change in the hereditary properties of the organism, which can occur in the course of its development only provided that, in this process, a change occurs in the character of the organism's metabolism. Accordingly, a microorganism cannot give rise to specific substances merely as the result of a single factor, i.e., the presence of organisms of another species, without the existence of a definite metabolic process that will come into being only in the course of several generations.

Evidently, in the cultivation of organisms on a starvation medium, the main role in the first stages of growth is played by products of the autolysis of the cells. The process involved here can be imagined roughly as follows:

In the process of utilization of the products of cell autolysis, the organism which finds itself in the more advantageous position develops to a predominant extent. In the course of the development of the culture, the newly formed cells are exposed to new and different conditions in the culture medium,

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conditions to which they are then able to adapt their own metabolism. Concomitant to the development of organisms of one species in the medium, the other species of cells can likewise exhibit life activity. It goes without saying that under these conditions, the struggle for survival between the different species plays an essential role.

In the course of this struggle for survival, which is connected with the development of the organisms, some of the cells of one species, having altered their metabolism to conform with the new culture conditions, are able to exercise their ability to form anti-bacterial substances in such a way as to cause lysis of the cells of the other species. The products of this cytotoxicity are then utilized as nutritive material by the active antagonist.

2. In the second chapter of the second section, which is devoted to an examination of forced antagonism between yeast cells and bacteria, Shiller directs his attention to the interrelationship between the smallpox virus and yeast cells. In this regard, he bases his work on the investigations of Hill'ber (4) and Vostroukhova and assumes that this is a typical instance of forced antagonism. Here, in his opinion, the virus plays the part of the active antagonist.

In one of Hill'ber's latest works, it is noted that between the virus of the variolus vaccine and the yeast *Saccharomyces* there exists a relationship of symbiosis. However, although this symbiotic interrelationship there exists as well, according to Hill'ber, an antagonistic relationship. But this antagonism consists of the fact that bacterial polysaccharides retard the development of some viruses. Hill'ber, in referring to the microphotographs of Besh'yan (1,45), also expresses the opinion that equine infectious anemia is associated with yeast cells, which act as carriers of the virus of equine infectious anemia.

All these data indicate that some viruses can be found in definite interrelationships with yeast cells, and that these interrelationships exist between them under natural conditions.

If we agree with Shiller's view that forced antagonism can take place only under conditions created by the experimenter, then there is no necessity to consider these interrelationships which occur under natural conditions and which exist without the interference of an experimenter as instances of forced antagonism.

However, is Shiller correct in assuming (p 38) that forced antagonism between microbes can occur only under conditions created by the experimenter, while natural antagonism occurs during the normal growth and reproduction of microbes in a pure culture? It seems to us that making a distinction between "forced" and "natural" antagonism on this basis is not quite reasonable. In fact, under natural conditions which are independent of the will of any experimenter, the occurrence of a mutual relationship is entirely possible between microorganisms wherein one of them is found to be more adaptable than the other and is able to utilize the cell contents of the other as nutrient material.

3. The fourth chapter of the second section attempts to prove the existence of the forced antagonism phenomenon between bacteria.

A study of intestinal microflora furnishes proof of the existence of forced antagonism in vivo under conditions when there is a copious food supply.

By feeding animals and men on lactose or dextrin, Shiller and his co-workers succeeded in obtaining a superior development in the intestines of *Bact. biridum* (a facultatively anaerobic variant of *Bact. acidophilum*). The

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author explains the predominance of *Bact. bifidum* in animal and human intestines under the given feeding conditions by the fact that this organism "finds itself in a superior position versus the other bacteria, so that it is easier for it to struggle with them, which it does by excreting substances that are bacteriolytic for them." (p 91) In other words, Shiller perceives here a typical instance of forced antagonism between *Bact. bifidum* and the accompanying microflora, which is mainly putrescent.

However, from our point of view, there has not been enough of that direct observation of the action of one of the microorganisms on the vital functioning of the other which, as the author so correctly indicates on p 86, is necessary in order to justify this conclusion.

The concept of forced antagonism, as understood by Shiller, assumes the utilization of one of the microorganisms by the other as food. Shiller has not established the existence of a process of this type involving intestinal microflora.

The inference about the existence of forced antagonism in the intestine is made merely on the basis of a supposition and is corroborated only by indirect evidence. But there are also reasons for making a different assumption.

Undoubtedly, there is an antagonism in the intestine between the lactic acid bacteria and the accompanying microflora. This had already been pointed out by I. I. Mechnikov. This antagonism, however, depends on the fact that the lactic acid bacteria, thanks to their own life activity, create conditions in the medium which are unfavorable for the microflora, namely, a change in the pH of the medium due to the formation of lactic acid. On the other hand, it has been demonstrated that lactic acid bacteria inhabiting the intestine can, in addition to forming lactic acid, develop specific antibacterial substances. This too was indicated by Mechnikov in his time. In any event, one can hardly speak here of a forced antagonism.

Shiller's theory of forced antagonism is completely substantiated by a whole series of persuasive examples so that, in order to prove it, there is no need to introduce facts which, from a theoretical point of view, give rise to doubt.

Shiller's book would have gained if the author had not so persistently drawn upon the microflora and virus examples above in order to support his theory.

Despite the indicated errors, the book, as a whole, will undoubtedly be of interest to theoretical and practical medical workers, microbiologists, and other biological specialists.

Microbiologists are faced with the task of discovering new antibiotic substances which will react against definite groups of microorganisms. This book can be of help to them in obtaining, by means of directed modification, controlled strains of microorganisms with specific properties.

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